

Longitudinal investigation of *Salmonella* spp. from farm to fork in the pig industry in Reunion Island

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Abstract

Salmonella is, after *Campylobacter*, the most reported zoonotic pathogen in Europe. Pork meat is frequently incriminated in salmonellosis. However, asymptomatic carrying of *Salmonella* by pigs makes its detection difficult. Despite its tropical situation, pig industry of Reunion Island is also concerned by this public health burden.

Our epidemiological study aims to identify the pathways of contamination along the pig chain industry. Three *Salmonella* positive farms were followed from the farrowing to the pork cuts. At farm level, *Salmonella* excretion was detected by individual samples of feces at birth, farrowing, post-weaning and fattening. During slaughtering, samples were taken from pigs entering and from carcasses after polishing, splitting and chilling. Caeca and pork cuts were also collected. Environmental samples of water, food and environment at farm and at different stages before slaughtering and cutting of the day were taken.

Among the 900 analysed samples, 229 isolates were collected and divided into 42 pulsotypes and 12 serotypes. *S. monovariant* 4,[5],12:i:-, *S. Rissen*, *S. Typhimurium* and *S. Livingstone* were predominant. Three different infection profiles were observed. At the nursing stage, piglets were *Salmonella* free. Infection occurred either after post-weaning (2/3) or during the fattening period (1/3). Truck and lairage are cross-contamination stages (100% are positive before pig loading). On the contrary, slaughterhouse is a decreasing contamination stage (equipment and pork cuts are *Salmonella* free). Farm surroundings play a major role in the reinfection of pigs like other animal productions.

This original investigation, made for the first time until now, give an accurate photography of *Salmonella* spreading along the chain.

Introduction

Salmonella is one of the main zoonotic pathogens in Europe. Pork meat is frequently incriminated in salmonellosis. Despite being highly studied in poultry production, epidemiological data are missing concerning *Salmonella* contamination of pork meat in Reunion Island. Limiting *Salmonella* contamination is dependent on identifying the source and ways of dissemination of *Salmonella* transmission at each stage during production and during slaughtering.

The aim of this study was to identify the pathways of contamination all along the chain industry of Reunion Island, from the farm to pork cuts.

Material and Methods

Three farrow-to-finish farms, previously detected *Salmonella* positive, were selected for this project. Farms were selected from different geographical locations in Reunion Island and with different environmental characteristics and different general husbandry practices. They were monitored from farrowing to the pork cuts. Three sows and 5 piglets by sow were selected from one batch per farm.

At farm level, *Salmonella* excretion was detected by individual samples of feces, after farrowing and throughout each growing stage. At slaughterhouse level, gauze swabs from pigs entering and from carcasses after polishing, splitting and chilling, caecal contents and pork cuts were sampled. Environmental samples were collected at both farm and slaughterhouse levels.

They were collected at each visit in farms and all along the slaughtering and cutting process at the beginning of the day, before the slaughter of the followed pig batch.

Environmental and feces samples were pre-enriched in buffered peptone water (BPW) in 1:10 sample/broth ratio. Disinfectant neutralizer was added in BPW broth (1:10) for samples collected after cleaning and disinfection. Environmental swabs and socks were pre-enriched with 150 and 300 ml of BPW respectively. After incubation at 37°C for 18h ± 2h, one ml and 3

drops of BPW broth were inoculated respectively to 10 ml of Müller–Kauffmann Tetrathionate-Novobiocin broth (MKTn) and on Modified Semisolid Rappaport Vassiliadis agar (MSRV) plate. Both were incubated respectively at 37°C for 24 h and 41.5°C for 24-48h. Samples of feed were analysed as described previously. However, selective enrichment was done with MKTn and Rappaport Vassiliadis Broth (RVB), incubated respectively at 37°C and 41.5°C for 24h. A loopful of selective enrichment in MKTn or RVB was then streaked on Xylose-Lysine-Desoxycholate Agar (XLD) and Rambach Agar. MSRV plates with characteristic halo of migration were streaked onto XLD and Rambach agar. After 24h at 37°C, four typical colonies per sample (one per way if possible) were biochemically confirmed as *Salmonella* and serotyped according to the Kauffman-White scheme.

Results

Amongst the 900 analyzed samples, 229 isolates have been collected. They were divided into 42 *Xba*I pulsotypes and 12 serotypes: monovariant of Typhimurium 4,[5],12:i:- (72), Rissen (60), Typhimurium (32), Livingstone (25), Weltevreden (12), Derby (10), London (8), Senftenberg (3), Newport (3), Bredeney (1), Blockley (1) and Give (1). Some serotypes were predominant in some farms: 4,[5],12:i:- in farm A and B, Rissen in farm B and Typhimurium / Livingstone in farm C. Distribution of pulsotypes in farm B from farrowing to pork cuts is illustrated in Figure 1.

Water, feed, cockroach and rodents samples return all negative.

Although floor of maternity and corridor were positive for *Salmonella*, neither sows nor piglets excreted *Salmonella* until the end of nursing (2/3).

Piglets started to excrete *Salmonella* a few weeks after the beginning of post-weaning. Pulsotypes were similar to the ones found in the room after cleaning and disinfection, and in corridor before the beginning of post-weaning stage (2/3).

Afterwards, until the end of pig fattening, pulsotypes were identical to the ones previously found during post-weaning or in farm environment (including other animal productions). For one of the 3 farms, pigs were excreting *Salmonella* at a late fattening stage whereas, in the other ones, the number of contaminated pigs decreased during the fattening period

Loading bay (2/3), truck (3/3) and lairage room at slaughterhouse (3/3) were contaminated with new pulsotypes. Before slaughtering and cutting, equipment and tools were negative except knives (1/3). However, this pulsotype was not detected in any other sample. At slaughterhouse, just prior to slaughter, pigs were carrying both previously detected pulsotypes and new ones. Some pulsotypes were detected from carcasses after polishing (3/14 for farm B) and after splitting (2/14 for farm B and 2/15 for farm C). However, all samples collected after splitting steps were negative.

Discussion

All samples of water, feed, rodents and cockroaches were *Salmonella* negative. However, *Salmonella* has ever been isolated from same types of samples (Davies *et al.*, 1997; Letellier *et al.*, 1999) as well as in flies (Khalil *et al.*, 1994), all collected from *Salmonella* positive farms. These findings may suggest that they play a role in the spread of *Salmonella*.

None of the sows and none of the piglets were shedding *Salmonella* during nursing. Kranker *et al.* (2003) showed that, although some sows were seropositive during nursing, none piglets were *Salmonella* positive. This finding suggests two explanations. The first one could be that sows play a minor role in *Salmonella* contamination of pigs during growing. And the second one that passive maternal immunity of sows may have protected pre-weaned pigs from *Salmonella* contamination (Wales *et al.*, 2009). Moreover, maternal antibodies secreted in colostrum and milk could protect piglets against colonisation by different *Salmonella* serotypes (Funk *et al.*, 2001).

No piglets were excreted *Salmonella* before weaning and pulsotypes isolated from piglets and from residual infection were similar. These results confirm that horizontal transmission may be due to residual contaminations (Kranker *et al.*, 2003; Wales *et al.*, 2009).

Contamination of pens in farms studied after cleaning and disinfection is not surprising. Several studies have shown that cleaning and disinfecting pig house is recognized as a challenge (Magistrali *et al.*, 2008; Wales *et al.*, 2009). Moreover, *Salmonella* can persist outside animals for days to months, and is able to adapt himself to host but also to farm environment (Berends *et al.*, 1996).

Salmonella was isolated from cleaned truck suggesting that procedures for cleaning and disinfecting was not enough efficient to eliminate *Salmonella*. Same findings were already shown by (Swanenburg *et al.*, 2001). Lairage room was also positive before arrivals of pigs. Similar pulsotypes were isolated from truck and lairage room before loading and further on back of pigs, from carcasses after polishing and splitting and from caecal contents. This confirms that transport and lairage are important stages of cross-contamination (Magistrali *et al.*, 2008; Bolton *et al.*, 2013). No *Salmonella* was detected after splitting. This suggests that slaughter process reduce *Salmonella* contamination, notably after chilling. However, prevalence after this step could be underestimated, because a formation of an ice layer on the carcasses after chilling (Swanenburg *et al.*, 2001). Despite no pork meat were *Salmonella* positive, we cannot conclude that there is not a risk for consumer.

Conclusion

This investigation allows us to identify sources of *Salmonella* contamination at production and slaughterhouse stage. However, characterisation of all isolates per samples could confirm ways of contamination highlighted or to identify new ones. Indeed, individuals may be infected simultaneously with different serotypes (Funk *et al.*, 2001). MLVA on *S. Typhimurium* and *S. 4,[5],12,i:-* is in progress and will clarify the ways of contamination, indeed, the origin of some pulsotypes have not yet been identified.

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	After C&D	Entering	Farrowing +4days	Weaning	After C&D	+ 2 weeks	End	After C&D	+ 1 mo.	+ 1 mo.	+ 1 mo.	End
Sows	/	-	-	-								
Pigs	/	-	-	-	/	X06	X06		X06	X06; X42; X43	X20	X06
Room	-	-	-	X26	X06	X06	X06	X03	X05; X06	X05	X12; X20	X06
Corridor	X29	/	/	/	X06	/	/	-	/	/	/	X06
Loading bay	/	/	/	/	/	/	/	/	/	/	/	X11
Adjacent environment	/	/	X42	X26	/	X42	/	/	X04	X28	-	/
Other productions	/	/	X10	-	/	/	-	/	X04; X06	X07	-	/

	Loading	Lairage	Polishing	Evisceration	Splitting	Chilling	1 st cutting	2 nd cutting
Truck ±	<u>X45</u>							
Lairage room ±		<u>X45</u> ; <u>X52</u>						
Back of pigs *		X06 ; X15; <u>X45</u> ; <u>X52</u>						
Equipment ±			-	X53 (knife)	-	/	-	-
Carcasses *			X20; X33; <u>X45</u>		X44	-		
Caeca *				X06 ; X15; X33; X42; <u>X45</u>				
Pork cuts *							/	-

Figure 1: Distribution of *Salmonella* pulsotypes detected along the investigation in farm B from farrowing to pork cuts
C&D: Cleaning and disinfection. /: not done. -: negative sample. *: sampled after step indicated in the arrow. ±: sampled before step indicated in the arrow. Pulsotypes X03, X04, X05, X06, X07, X10, X12, X20 are S. 4,[5],12,i:-; X15, X26, X28 and X29 are S. Typhimurium; X33 is S. Rissen; X42, X43 and X44 are S. Livingstone; X45 is S. Derby; X52 is S. London and X53 is S. Bredeney. X06 (in bold) is a predominant pulsotype. X45 and X52 (underlined) are pulsotypes identified from truck and lairage room and detected on carcasses.